

AperTO - Archivio Istituzionale Open Access dell'Università di Torino

Contribution of visceral fat and hepatic fat to metabolic derangements and liver damage in NAFLD patients.

This is the author's manuscript

Original Citation:

Availability:

This version is available <http://hdl.handle.net/2318/130535> since 2017-02-10T12:05:14Z

Published version:

DOI:10.1016/S1590-8658(13)60010-9

Terms of use:

Open Access

Anyone can freely access the full text of works made available as "Open Access". Works made available under a Creative Commons license can be used according to the terms and conditions of said license. Use of all other works requires consent of the right holder (author or publisher) if not exempted from copyright protection by the applicable law.

(Article begins on next page)

fibroblasts (CAF). CAF are important determinants of cancer invasiveness. We have recently shown that CAF are recruited through a Platelet-derived Growth Factor (PDGF)-D-mediated paracrine mechanism originating from CCA cells. Herein, we aimed to understand the downstream effectors of PDGF-D signaling and the cellular mechanisms by which PDGF-D promotes the recruitment of CAF.

Methods: In human fibroblasts isolated from liver explants, we evaluated the *in vitro* effects of increasing doses of rhPDGF-D (0.1, 1, 10, 100 ng/ml) on: a) cell proliferation (MTS), b) cell migration (Boyden chambers), c) ERK1/2 expression (Western blotting), d) small GTPase (RhoA, Rac1, Cdc42) activities (G-LISA), before/after treatment with Imatinib (1 μ M), a PDGFR β antagonist. Fibroblast migration induced by rhPDGF-D 100 ng/ml was also evaluated after selective inhibition of RhoA (Y-27632, 10 μ M), Rac1 (NSC23766, 75 nM) and Cdc42 (CASIN, 5 μ M).

Results: Administration of rhPDGF-D significantly stimulated fibroblast proliferation only at high doses (10 ng/ml, 100 ng/ml). In contrast, rhPDGF-D induced a strong dose-dependent stimulation of fibroblast migration, starting at 0.1 ng/ml and increasing by nearly $\times 10$ the controls at 100 ng/ml ($p < 0.0001$). Both effects were inhibited by Imatinib. Similarly to fibroblast proliferation, PDGF-D induced a significant increase of p-ERK1/2 only at the highest doses. Conversely, PDGF-D induced a clear dose-dependent, Imatinib-inhibitable, linear increase for Rac1 and Cdc42 activities. RhoA was activated only at the highest doses. All chemical inhibitors of Rho GTPases induced a significant reduction in fibroblast migration (35% for Cdc42, 60% for Rac1, $p < 0.001$), that was completely abrogated by the combined treatment (Y-27632+NSC23766+CASIN).

Conclusions: PDGF-D secreted by CCA cells, stimulates PDGFR β expressed by fibroblasts, and by activating Rho GTPases (particularly Rac1 and Cdc42), stimulates cell motility, resulting in CAF recruitment. Targeting PDGF-D-mediated cross-talk between cancer cells and CAF at the level of PDGFR β or Rho GTPases may offer a novel therapeutic approach in CCA.

OC-07

Primary biliary cirrhosis and metabolic syndrome

N. Cazzagon¹, I. Franceschet¹, F. Canesso¹, L. Salmaso², A. Floreani¹

¹Department of Surgery, Oncology and Gastroenterology; ²Department of Management and Engineering, University of Padova, Padova, Italy

Background: PBC is characterized by a long natural history and a low incidence of cardiovascular events despite high cholesterol serum levels. However, the role of metabolic conditions (obesity, hypertension, insulin resistance) eventually associated to PBC has not been analyzed.

Aim: To assess the influence of metabolic syndrome (MS) on the response to UDCA and the survival in PBC patients.

Methods: The historic database collection (1975–2011) was used. The mean follow up was 123 months, (range 6–425 months). All patients were treated with UDCA (15 mg/kg/day). Hypercholesterolemia was treated with statins or fibrates when total cholesterol was > 240 mg/dl. Responders to UDCA were defined subjects who reached at least a 40% decrease in alkaline phosphatase after 1 year. MS was defined MS according to the American Heart Association criteria. Survival was analyzed with Kaplan-Meier curves.

Results: A total of 171 PBC patients were considered eligible for the study; 55 of them (32.1%) at presentation fulfilled the criteria for MS. LFTs and Mayo score were comparable in patients with MS and in those without MS; only GGT resulted significantly higher in the group with MS (243 vs. 159 IU/ml, $p = 0.038$). Histological stages and fibrosis score were similar in the two groups at baseline. The occurrence of cardiovascular events during the follow-up was significantly higher in patients with MS ($p < 0.01$). The response to UDCA was higher in the group without metabolic syndrome ($p < 0.05$). Kaplan-Meier curves were similar until a 200 month interval, thereafter survival was worse in the group with MS, but the difference was not statistically significant.

Conclusion: MS when associated to PBC should be carefully treated, due to the risk of cardiovascular events and the reduced response to UDCA.

OC-08

Dysbiosis contributes to liver fibrosis through bacterial translocation and elevated endotoxemia by increasing Gram- abundance

S. De Minicis, C. Rychlicki, L. Agostinelli, S. Saccomanno, C. Candelaesi, L. Trozzi, M. Marzoni, A. Benedetti, G. Svegliati Baroni

Department of Gastroenterology, Università Politecnica delle Marche, Ancona, Italy

Introduction: Non-alcoholic fatty liver disease may lead to hepatic fibrosis. Dietary habits affect gut microbiota; while endotoxins produced by Gram- bacteria stimulate hepatic fibrogenesis. However, the mechanisms involved in the process of liver damage and the potential effect of microbiota is still unknown.

Aim: To analyze whether microbiota composition and bacterial translocation may interfere with liver fibrogenesis.

Material, methods, and results: Mice were treated with control (CTRL) or high fat (HFD) diet for 1 month and subsequently subjected to either BDL (2 weeks) or i.p. CCl₄ (3 weeks). Microbiota transplantation was obtained by oral gavage of previously gut-sterilized mice by antibiotic cocktail (Neomycin, Ampicillin, metronidazole, vancomycin). mRNA for collagen $\alpha 1(I)$ and α SMA, collagen deposition (Sirius Red), hydroxyproline content, were increased in the HFD-BDL mice versus CTRL-BDL mice, while no differences were observed between CTRL-CCl₄ mice and HFD-CCl₄ mice. Culture of mesenteric lymphnodes showed higher density of infection in HFD-BDL mice versus CTRL-BDL mice, suggesting higher bacterial translocation rate. No evidence of bacterial translocation was observed in CCl₄-treated mice. HFD-BDL mice showed the highest reduction in occludin mRNA expression, suggesting increased intestinal permeability. Pyrosequencing analysis revealed a condition of dysbiosis characterized by an increase of Gram- vs. Gram+ bacteria, a reduced ratio between Bacteroidetes and Firmicutes and, more specifically, a dramatic increase of the Gram- proteobacteria in HFD-BDL versus CTRL-BDL mice. Inflammasome expression was increased in fibrotic liver, but significantly reduced in the gut. Furthermore, microbiota transplantation revealed more liver damage in the chimeric mice treated with CTRL diet but receiving the microbiota of HFD-treated mice.

Conclusions: By increasing the percentage of Gram- producing endotoxins, different dietary habits lead to a pro-fibrogenic effect in the course of chronic hepatic liver damage. Bacterial translocation, altered intestinal permeability and dysbiosis are needed by hepatic steatosis to act as co-factors in chronic liver diseases.

OC-09

Contribution of visceral fat and hepatic fat to metabolic derangements and liver damage in NAFLD patients

L. Mezzabotta¹, E. Vanni¹, C. Rosso¹, S. Carenzi¹, R. Gambino², M. Gaggini³, D. Lucarelli⁴, R. Faletti⁴, M. Cassader², A. Smedile¹, M. Rizzetto¹, A. Gastaldelli³, E. Bugianesi¹

¹Division of Gastroenterology and Hepatology, Department of Medical Sciences, San Giovanni Battista Hospital, University of Torino, Torino, Italy; ²Lab. of Diabetology, Department of Medical Sciences, San Giovanni Battista Hospital, University of Torino, Torino, Italy; ³Cardiometabolic risk unit, Institute of Clinical Physiology, CNR, Pisa, Italy; ⁴Radiology, San Giovanni Battista Hospital, University of Torino, Torino, Italy

Background: Both hepatic (HF) and visceral fat (VF) are involved in the onset and progression of metabolic syndrome and NAFLD. However, their relative role is still debated.

Aim: To determine the relative contribution of HF and VF to both metabolic derangements and histological liver damage in NAFLD patients.

Methods: In 22 non-diabetic, non-dyslipidaemic patients with biopsy-proven NAFLD we measured whole abdomen VF by MRI, fasting endogenous glucose production (EGP) and lipolysis by tracer infusion, peripheral insulin resistance (IR) as HOMA, Hepatic-IR (EGP*insulin) and Adipo-IR (FFA*Insulin). Hepatic histology was scored according to Kleiner, HF was assessed as percentage of liver fat.

Results: VF increased with BMI ($r = 0.54$, $p < 0.004$) and HF ($r = 0.43$, $p < 0.03$),

whereas HF was not correlated with BMI. Only HF, but not VF, was associated with circulating FFA levels ($r=0.51$, $p<0.01$), and Adipo-IR ($r=0.41$, $p<0.05$), while no correlation was found between either LF% or VF with Hepatic-IR or HOMA.

Patients with NAS score ≥ 4 (vs. NAS 0–3) had increased VF (3.9 ± 0.7 vs. 2.8 ± 0.4 kg), FFA concentrations (525 ± 52 vs. 804 ± 98 mmol/l), peripheral IR (HOMA: 3.7 ± 0.5 vs. 2.8 ± 0.8), Hepatic-IR (169 ± 23 vs. 124 ± 32), and Adipo-IR (13.2 ± 2.5 vs. 6.3 ± 1.8) (all $p<0.05$). Considering only ballooning and lobular inflammation in the NAS score, subjects with a composite score >2 had an impaired lipolysis suppression.

Patients with fibrosis vs. those without fibrosis, had increased VF (3.9 ± 0.6 vs. 2.7 ± 0.7 kg, $p<0.03$), but not HF, and no differences were found in the indexes of IR.

Conclusions: In NAFLD subjects, hepatic fat is associated with metabolic derangements and IR, but Adipo-IR and visceral fat accumulation appear to provide a major contribution to liver damage.

Funding from the FP7/2007-2013 under grant agreement n°Health-F2-2009-241762, for the FLIP project.

OC-10

Hepatocyte-derived microparticles released in blood as biomarkers of NASH development

D. Povero^{1,2}, A. Eguchi¹, C. Johnson¹, A. Wree¹, M. Lazic¹, M. Parola², A.E. Feldstein¹

¹Department of Pediatrics, University of California San Diego (UCSD), La Jolla, CA, USA; ²Department of Clinical and Biological Sciences, Unit of Experimental Medicine, University of Torino, Italy

Introduction and aim: At present there is a lack of effective treatments and noninvasive diagnostic markers for nonalcoholic steatohepatitis (NASH). We have recently demonstrated that hepatocyte-derived microparticles (MPs) are critical signals contributing to angiogenesis and liver damage in NASH (Presidential Plenary, AASLD 2012). Here we tested the hypothesis that circulating hepatocyte-MPs are novel targets for noninvasive NASH monitoring.

Methods: Male C57BL/6 mice were placed on Choline-Deficient L-Amino Acid (CDAA) diet, Choline-Supplemented L-Amino Acid (CSAA) or regular-diet (RD) for 4 and 20 weeks (early stage and established NASH, respectively). Circulating MPs were isolated from platelet-free plasma, detected by flow cytometry and extensively characterized by electron microscopy in liver tissue and circulation, dynamic light scattering and by LC MS/MS proteomic analysis. Liver specimens were collected and used for histological, biochemical, and molecular analysis of steatosis, inflammation, angiogenesis, fibrosis and cell death.

Results: MPs circulating levels were significantly increased only in mice with established NASH (20 wks CDAA diet: $304,400$ MPs/mL vs. 20 wks CSAA $34,300$ MPs/mL vs. RD $2,000$ MPs/mL, $p<0.0052$). The increase was time-dependent and MPs blood levels strongly correlated with histological features of liver damage, in particular with fibrosis, as determined by morphologic quantification of Sirius Red staining ($r=0.736$; $p<0.0002$), and cell death determined by TUNEL assay ($r=0.804$; $p<0.0001$). We next characterized the antigenic composition of circulating MPs using a comprehensive proteomic approach by LC-MS/MS analysis. A gene ontology analysis of the proteins identified 26.5% plasma membrane proteins, 58.8% cytoplasmic proteins, 8.8% nuclear proteins and 5.9% extracellular proteins. Analysis of the molecular function of these proteins demonstrated that 35.3% were enzymes, 41.2% were cytoskeleton or vesiculation proteins, 2.9% nucleosome proteins and 5.9% ribonucleoproteins.

Conclusion: Our data identified circulating MPs with a unique antigenic composition as potential novel biomarkers for noninvasive diagnosis of NASH.

OC-11

PNPLA3 GG genotype is associated with carotid atherosclerosis in patients with non-alcoholic fatty liver disease

S. Petta¹, G. Parrinello², V. Di Marco¹, A. Licata¹, C. Cammà¹, M.R. Barcellona¹, D. Cabibi³, S. Grimaudo¹, M.R. Pipitone¹, D. Torres², G. Marchesini⁴, G. Licata², A. Craxi¹

¹Sezione di Gastroenterologia, Di.Bi.M.I.S, Università di Palermo, Italy;

²Dipartimento Biomedico di Medicina Interna e Specialistica (Di.Bi.M.I.S), Università di Palermo, Italy; ³Dipartimento di Patologia umana, Università di Palermo, Italy; ⁴Dipartimento di Medicina e Gastroenterologia, "Alma Mater Studiorum", Università di Bologna, Italy

Background and Aims: There is evidence that patients with non-alcoholic fatty liver disease (NAFLD) are at high cardiovascular risk and that the prevalence and the severity of NAFLD are also driven by genetic background. In NAFLD patients we tested the association of carotid atherosclerosis with clinical, genetic and histological factors.

Materials and Methods: We assessed anthropometric, metabolic and histological data (Kleiner score) in 162 consecutive biopsy-proven NAFLD patients. Intima-media thickness (IMT) and carotid plaques, defined as focal thickening of >1.3 mm at the level of common carotid artery, were evaluated using ultrasonography. IL28B rs12979860 C>T, PNPLA3 rs738409 C>G, GCKR rs780094 C>T, LYPLAL1 rs12137855 C>T, and NCAN rs2228603 C>T single nucleotide polymorphisms were also assessed.

Results: Carotid plaques were found in 59 (36.4%) cases, and in particular, in 32% PNPLA3 CC/CG vs. 52.9% PNPLA3 GG patients ($p=0.02$). Multivariate logistic regression analysis showed that older age (OR 1.102), female gender (OR 2.476), type 2 diabetes (OR 4.061), and PNPLA3 GG genotype (OR 2.679) were independently linked to the presence of carotid plaques. In patients aged <50 years, 8/77 cases with PNPLA3 CG/CG genotype (10.9%) had carotid plaques, vs. 6/15 (40%) with PNPLA3 GG genotype ($p=0.02$). By contrast, in patients ≥ 50 years the prevalence of carotid plaques was similar in subjects with PNPLA3 CC/CG and GG genotype (33/55, 60% vs. 12/19, 63%; $p=0.75$). Similarly, PNPLA3 CC/CG cases aged <50 had a lower IMT than PNPLA3 GG patients (0.75 ± 0.17 vs. 0.85 ± 0.25 mm; $p=0.05$), while this difference was not observed in patients aged ≥ 50 (0.93 ± 0.25 vs. 1.00 ± 0.26 mm; $p=0.32$).

Conclusion: PNPLA3 GG genotype is associated with a high risk of early carotid atherosclerosis in NAFLD.

OC-12

P53 and hepatic lipid metabolism: a new interesting connection

M. Gori¹, B. Barbaro¹, M. Arciello¹, A. Longo¹, C. Viscomi¹, C. Balsano^{1,2}

¹Laboratory of Molecular Virology and Oncology, Andrea Cesalpino Foundation, Rome, Italy; ²Institute of Biology and Molecular Pathology (IBPM)-National Research Council (CNR), Rome, Italy

Introduction: Nonalcoholic fatty liver disease (NAFLD) is the most common form of chronic liver disease worldwide, ranging from simple steatosis to steatohepatitis which may progress to cirrhosis, and hepatocellular carcinoma. However, the molecular mechanisms underlying NAFLD development have not been completely characterized. Recently, the p53 tumor suppressor protein has gained attention as a modulator of cellular metabolism, responding to several stress conditions, but its role in the progression of NAFLD remains poorly understood. We designed an *in vitro* model of NAFLD to analyze how different forms of p53 may affect the metabolic stress response induced by free fatty acid (FFAs) overloading.

Materials and methods: Three cell lines carrying different forms of p53: HepG2 (p53 wt), Huh7.5.1 (mutated-p53) and Hep3B (p53 knockout), were cultured for 14 h in a medium containing oleic and palmitic acid (molar ratio 2:1, respectively) at a final concentration of 1 mM, the most abundant FFAs in western diets. Intracellular lipid accumulation and cell viability were assessed by AdipoRed and MTS assay, respectively. Gene and protein expression profiles of p53 and lipid metabolism-related genes were evaluated by qRT-PCR and Western blotting.

Results: In the three cell lines the FFA treatment was not cytotoxic and